UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/990,705	11/21/2001	Judith K. Gwathmey	JGT-004	3899

37462 7590 04/26/2004

LOWRIE, LANDO & ANASTASI
RIVERFRONT OFFICE
ONE MAIN STREET, ELEVENTH FLOOR
CAMBRIDGE, MA 02142

EXAMINER	
AFREMOVA, VERA	

ART UNIT	PAPER NUMBER
1651	

DATE MAILED: 04/26/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/990,705	GWATHMEY ET AL.	
	Examiner	Art Unit	
	Vera Afremova	1651	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *30 January 2004*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☐ Claim(s) *1-26* is/are pending in the application.

4a) Of the above claim(s) *14-26* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) *1-13* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Status of claims

Claims 1-13 as amended {1/30/2004} are under examination in the instant office action.

Claims 14-26 were withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to nonelected inventions without traverse {3/18/2003}. A complete reply to the final rejection must include cancelation of nonelected claims or other appropriate action (37 CFR 1.144) See MPEP § 821.01.

Claim Rejections - 35 USC § 112

Claims 1-13 as amended remain rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1 and 13 remain indefinite and unclear with regard to concentration or amounts of calcium chloride. The claims are rendered indefinite by a combination of the phrases "decreasing" and "increasing" with the identical amounts 1-2 μM for both "decreasing" and "increasing" concentrations. On one hand, the claimed method requires to decrease calcium amounts for cell dissociation/isolation and to increase calcium amounts for restoration of cellular function of the isolated cardiomyces. However, the amounts of calcium as claimed are identical. Thus, the concept of manipulating calcium amounts, if there are any intended changes, is unclear as claimed.

Furthermore, with regard to the phrase "modified Earle's" (claims 1, 2 and 13), it remains unclear what ingredients and amounts are claimed. The "Earle's" complete medium contains

large amounts of calcium and, thus, it is uncertain what amounts of calcium are intended for starting or final points at "increasing" as claimed (claims 1 and 13).

In claims 12 and 13 the claimed amount of ascorbic acid fails to indicate for what medium volume it is intended. Thus, these claims are indefinite because it is uncertain whether the same amount of 8.8 mg of ascorbic acid is used regardless the amounts of other components theirs amounts in the claimed method.

Claim Rejections - 35 USC § 103

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Claims 1-13 as amended remain rejected under 35 U.S.C. 103(a) as being unpatentable over Kruppenbacher et al. (IDS reference C4) taken with the ATCC catalogue and Kang et al. (IDS reference C3) as explained in the prior office action and for the reasons below.

Claims are directed a method of isolating adult cardiac cells comprising step of obtaining a tissue sample from a subject, step of successively exposing the tissue to a first solution with decreasing amounts of $CaCl_2$, wherein the fist solution comprises NaCl, HEPES, $MgC1_2$, KC1 and sugar and has pH about 7.4; step of disassociating the tissue with an enzyme solution; step of repeatedly resuspending the disassociated tissue into second solution with increasing amounts of $CaCl_2$, wherein the second solution comprises L-glutamine, sodium bicarbonate, sodium pentothenate, creatine, taurine, ascorbic acid, HEPES and antibiotic and has pH 7.4 in order to obtain the isolated cells. Some claims are further drawn additional step of resuspending the isolated cells. Some claims are further drawn to incubating isolated cells in a mixture of carbon

dioxide and air at temperature 37 degree C. Some claims are further to exposing the tissue to a first solution at 37 degree C at 4 ml/min for 3 minutes. Some claims are further drawn to the use of digestive enzyme protease or collagenase in the enzyme solution a method of isolating cells. Some claims are further drawn to particular concentration of the ingredients in the first and in the second solutions. Some claims are further drawn to the use of particular ingredient in the enzyme solution such as NaCl, HEPES, $MgCl_2$, KCl and glucose at particular concentrations.

The cited references are relied upon as explained in the prior office action and repeated herein.

Kruppenbacher et al. discloses a method of isolating adult cardiomyocytes wherein the method comprises step of obtaining a heart tissue sample from a subject (page 133, col. 1, lines 14-16); step of successively exposing the tissue to a first solution or buffer with decreasing amounts of $CaCl_2$ from 25 μM (page 133, col. 1, line 24) to zero as in water (page 133, col. 1, lines 33-36) wherein the first solution of buffer comprising NaCl, Mg salt, KCl and glucose (page 133, col. 1, lines 20-22); step of disassociating the tissue with an enzyme solution comprising digestive enzymes protease (trypsin) and collagenase (page 133, col. 1, line 25) as well as other components of the first buffer solution including NaCl, HEPES, $MgCl_2$, KCl and glucose (page 133, col. 1, lines 20-22); step of repeatedly resuspending the disassociated tissue and cells into second solution or buffer with increasing amounts of $CaCl_2$ such as 0.2/0.2/1.0 mM and finally in the M 199 culture medium comprising salts, sodium bicarbonate, creatinine, taurine and antibiotic (page 133, col. 2, lines 5-12). The cited reference discloses incubating isolated cells in a mixture of carbon dioxide and air at temperature 37 degree C (col. 3, line 6).

The cited reference discloses step of exposing the tissue to a first solution at 37 degree C at 4 ml/min for 20 minutes (page 13, col. l, line 26).

The cited reference by Kruppenbacher et al does not disclose the use of buffer HEPES in the first solution but it clearly teaches the use of a buffer that is a physiologically acceptable buffer for cardiomyocytes and that is reasonably expected to have a neutral pH of about 7.4. The periods of the tissue exposure to the first solution in the method of the cited reference appears to be different but the claimed method does not indicate how many steps and how much tissue materials have been used in the method for isolating cells from the tissue.

The cited reference by Kruppenbacher et al. is silent with regard to the "modified Earl's" medium ingredients. However, the method by Kruppenbacher encompasses the use of the medium M199. The medium M 199 is known to comprise ingredients required by the presently claimed method including ascorbic acid and sodium pentothenate, for example: see the ATCC catalogue at page 522.

Furthermore, the reference by Kang et al. is relied upon to demonstrate that solutions suitable for isolating, culturing and maintaining function of cardiomyocytes comprise the presently claimed ingredients including buffer HEPES , NaCl, HEPES, $MgCl_2$, KCl and glucose at the presently claimed amounts (page 9887, col. 1, lines 1-2).

Although the cited reference by Kruppenbacher et al. might not clearly disclose the identical concentrations of all ingredients in the solutions and media as required by the claimed method, however, the use of particular amount of nutrients in the cell culture media is reasonably expected to be adjustable with regard to physiological requirements of a particular cell or tissue culture system.

Therefore, it would have been obvious to one having ordinary skill in the art at the time the claimed invention was made to practice a method of isolating adult cardiac cells by dissociating cardiac tissue and cells with low or decreasing amounts of calcium and further with the increasing amounts of calcium to restore cellular function as taught by the reference by Kruppenbacher et al with a reasonable expectation of success in isolating viable and active cells from the tissue as taught by the reference by Kruppenbacher et al. The cited method Kruppenbacher et al. is substantially similar, if not identical, to the presently claimed method as explained above. It is considered to be within the purview of one of skill in the art to adjust interval of incubation, pH or amounts of nutrients with regard to a particular cell or tissue culture system. One of skill in the art is would have been motivated to do so for the expected benefits in maximizing effects related to the cell survival, activity and function.

Thus, the claimed invention as a whole was clearly *prima facie* obvious, especially in the absence of evidence to the contrary.

The claimed subject matter fails to patentably distinguish over the state art as represented be the cited references. Therefore, the claims are properly rejected under 35 USC § 103.

Response to Arguments

Applicants' arguments filed 1/30/2004 have been fully considered but they are not persuasive.

With respect to the claim rejection under 35 U.S.C. 112 applicants argue that the fact that the amount of calcium are increased and decreased between one repeat and the next (response page 8, par. 4) is claimed. Yet, the claimed method requires the use of the same amounts as

claimed for the first and second solutions. The starting and final amounts, if different, are not clearly delineated in the claimed method. Applicants' generic disclosure is uncertain and/or broad with respect to the calcium concentration (pages 5-6). The solutions that are used in the particular examples comprise various ingredients and some of the ingredients are not identical to the ingredients in the presently claimed solutions (pages 11-12). Thus, no claim amendment is suggested in the instant office action because it is uncertain what is claimed, intended and/or argued.

With respect to the claim rejection under 35 U.S.C. 103 applicants appear to argue that the instant method is drawn to isolating of **adult** cardiac cells (response page 9, par. 3). However, the reference by Kruppenbacher clearly discloses isolation of cardiac cells from adult animals (see page 133, col. 1, par. 2, line 2). The reference by Kruppenbacher clearly teaches identical concept of decreasing calcium amounts for dissociation of cardiac tissues and cells and further recalcification for restoration of the isolated contracting cardiomyocytes.

Applicants appear to argue that the precise ingredients and theirs amounts are critical to success and that the reference by Kruppenbacher teaches away from the medium modifications. However, the method as disclosed by Kruppenbacher encompasses the use of identical culture medium as the applicants' medium 199 (see instant specification page 11, line 13) and the use of same or similar salt solutions with decreasing and increasing calcium amounts. Although the cited reference suggests that incorporation of growth factors might not be beneficial for a long-term storage of cardiomyocytes, the claimed method is not drawn to the cell storage. Furthermore, the substitution of one buffering agent for the other buffering agent (Hepes and

phosphate) wherein both agents provide for the same pH is not reasonably expected to change the material and/or functional differences, if any, in the methods as disclosed and as claimed.

Thus, applicant's arguments fail to comply with 37 CFR 1.111(b) because they amount to a general allegation that the claims define a patentable invention without specifically pointing out how the language of the claims patentably distinguishes them from the references.

Applicant's arguments do not comply with 37 CFR 1.111(c) because they do not clearly point out the patentable novelty which he or she thinks the claims present in view of the state of the art disclosed by the references cited or the objections made. Further, they do not show how the amendments avoid such references or objections.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Vera Afremova whose telephone number is (571) 272-0914. The examiner can normally be reached from Monday to Friday from 9.30 am to 6.00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Michael Wityshyn can be reached at (571) 272-0926.

The fax phone number for the TC 1600 where this application or proceeding is assigned is (703) 872-9306.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (571) 272-1600.

Vera Afremova

AU 1651

April 20, 2004

V. Afremova

VERA AFREMOVA

PATENT EXAMINER

SANDRA E. SAUCIER
PRIMARY EXAMINER